RESEARCH ARTICLE

Timing of gene expression from different genetic systems in shaping leucine and isoleucine contents of rapeseed (*Brassica napus* L.) meal

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Abstract

Experiments were conducted on rapeseed (*Brassica napus* L.) using a diallel design with nine parents: Youcai 601, Double 20-4, Huashuang 3, Gaoyou 605, Zhongyou 821, Eyouchangjia, Zhong R-888, Tower and Zheshuang 72. The seed developmental process was divided into five stages, namely initial (days 1–15 after flowering), early (days 16–22 after flowering), middle (days 23–29), late (days 30–36), and maturing (days 37–43) developmental stages. The variation of dynamic genetic effects for leucine and isoleucine contents of rapeseed meal was analysed at five developmental stages, across different environments using the genetic models with time-dependent measures. The results from unconditional and conditional analyses indicated that the expression of diploid embryo, cytoplasmic and diploid maternal plant genes were important for leucine and isoleucine contents at different developmental stages of rapeseed, particularly at the initial and early developmental stages. Among different genetic systems, nutrition quality traits were mainly controlled by the accumulative or net maternal main effects and their GE interaction effects, except at maturity when the net diploid embryo effects were larger. The expression of genes was affected by the environmental conditions on 15, 22, 29 or 36 days after flowering, but was more stable at mature stage. For the isoleucine content the narrow-sense heritabilities on 15, 22, 29, 36, and 43 days after flowering were 43.0, 65.7, 60.1, 65.5 and 78.2%, respectively, while for the leucine content the corresponding narrow-sense heritabilities were relatively smaller. The interaction heritabilities were more important than the general heritabilities at the first three developmental times. The improvement for isoleucine content could be achieved by selection based on the higher narrow-sense heritabilities. Various genetic systems exhibited genetic correlations among the developmental times or leucine and isoleucine contents. A simultaneous improvement of leucine and isoleucine contents seems possible because of the significant positive genetic correlation components from different genetic systems at different developmental times.

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Introduction

Oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops all over the world. On average, rapeseeds possess 22.5% crude protein in addition to 43.5% crude fat (Becker *et al.* 1999). Rapeseed meal, a byproduct of rapeseed oil milling contains amino acids that are an important source of nutrition for animals (Goding *et al.* 1972; Huisman and Tolman 1990). The nutrition value of rapeseed is not less than that of soybean (Josefesson and Muhlenberg 1968; Simbaya *et al.* 1995). Though the economic value of rapeseed is mainly determined by the seed oil content, increasing of seed protein content and essential amino acids are important breeding objectives in many breeding programmes (Hom *et al.* 2007). The genetic analysis for protein content of rapeseed has been documented using traditional statistical analysis (Wu *et al.* 2005). Previous studies have revealed that protein content in rapeseed is an important quantitative trait controlled by embryo nuclear genes (Brandle and McVetty 1988), cytoplasmic genes (McVetty and Pinnisch 1994), or diploid maternal plant nuclear genes (Grami and Stefansson 1977). It has been shown that the protein content and some amino acids, such as glutamic acid (Glu), glycine (Gly) and arginine (Arg) contents in rapeseed meal, are simultaneously governed by the genetic main effects from embryo (cotyledon), cytoplasmic, diploid maternal plant genetic system and their genotype × environmental (GE) interaction effects (Ren *et al.* 2005; Wu *et al.* 2005). However, in previous reports, the genetic effects were only analysed using

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phenotypic values of the traits at maturity and hence incapable of providing valuable information on the expression of genes at any one particular developmental stage.

The formation of protein and oil contents in the seed is an outcome orchestrated by the temporal and spatial expression of genes after pollination through a series of developmental steps (Variath *et al.* 2009). Therefore, variation in genetic effects and gene expression for amino acid contents in rapeseed meal might exist at different developmental stages.

Most quality traits are the results of gene expression along with seed development. The developmental genetic mechanisms of seed quality traits are difficult to study by using the conventional statistical analysis (Henderson 1985; Cowley and Atchley 1992; Atchley *et al.* 1994). Genetic models and methods of statistical analysis proposed by Zhu (1995) could be effectively applied to analyse the dynamic genetic effects at different developmental times or at any one specific period ($t-1\rightarrow t$). These models have been successfully used to study the developmental genetics and net genetic effects of the quantitative quality traits in rice (Shi *et al.* 2002a, b, 2005, 2006; Zhang *et al.* 2004). To date, the development genetic analysis of oil and protein in the seed has been only documented for rapeseed using one-year data (Variath *et al.* 2009). Leucine and isoleucine are two important essential amino acids that are closely related to the quality of rapeseed meal. Little is known about the expression of dynamic genes at various developmental stages and the influence of GE interaction effects on the leucine or isoleucine content in rapeseed under different environments.

In the present study, the developmental genetic behaviour of leucine and isoleucine contents of rapeseed meal was investigated using conditional and unconditional genetic models and statistical methods in different environments. The net genetic effects at different developmental times from flowering/impregnation to maturity and the conditional gene expression at one special developmental stage were analysed; the difference of dynamic gene expression at different developmental times was discussed for leucine and isoleucine contents of rapeseed meal. In addition, general and interaction heritability from different genetic systems at different developmental times and the genetic developmental correlations between leucine and isoleucine contents were analysed.

Materials and methods

Plant material

The experiments were conducted in 2006–2007 and 2007–2008, using a diallel design for nine parents: Youcai 601, Double 20-4, Huashuang 3, Gaoyou 605, Zhongyou 821, Eyouchangjia, Zhong R-888, Tower and Zheshuang 72. Parents and F_1 were sown on 9 October 2006 or 13 October 2007 at experimental farm of Zhejiang University (120°11′27″E, 30°16′28″N), and the 38-day-old seedlings were individually transplanted in the field at a spacing of 35 cm × 30 cm. Each parent and F_1 plot consisted of 32 plants. The experiment was laid out in a randomized block design with two replications. The date on which each flower opened was noted. The F_1 seed samples for each developmental time were derived by crossing females to males during the same growing season by hand crossing to avoid contamination. Seed sampling was carried out on 15, 22, 29, 36 and 43 days after flowering (DAF). From the centre of each plot, 12 plants were selected for seed samples of parents and F_2, and several inflorescences of these plants were randomly set in the photic bag before anthesis. The seed developmental process was divided into five stages, namely initial (days 1–15 after flowering), early (days 16–22 after flowering), middle (days 23–29), late (days 30–36), and maturing (days 37–43) stages.

Determination for the leucine and isoleucine content of rapeseed meal

The leucine and isoleucine contents of the rapeseed meal at different developmental times were determined using a NIR Systems model 5000 near-infrared reflectance spectroscope (NIR Systems, Silver Spring, USA). The NIR equations for leucine and isoleucine contents of the rapeseed meal with the coefficient of determination (it is described by the r^2 or RSQ) of 0.966 and 0.914, and their corresponding standard error of calibration (SEC) were 0.094 and 0.085, respectively (Chen *et al.* 2011b). About 3 g of each intact sample were scanned in a 36 mm inner-diameter ring cup (Shenk and Westerhaus 1993). All samples from each parent, F_1 and F_2 were measured from two replications.

Statistical analysis methods

The genetic models and statistical procedures for quantitative traits of diploid plant seeds (Zhu and Weir 1994; Zhu 1995) were used to estimate the unconditional and conditional genetic main effects and GE interaction effects at different developmental times/stages. For a mating design with a set of inbred lines, the generations mean (y_{hijk}) of mating type k from maternal line i and paternal line j in block l of environment h were partitioned as,

$$y_{hijkl} = \mu + E_h + G_{ijk} + GE_{hijk} + B_{l(h)} + e_{hijkl}.$$

Where μ, the population mean (fixed effects); E_h, the environmental effect (fixed); G_{ijk}, genotypic effect (random); GE_{hijk}, genotype × environment interaction effect (random); $B_{l(h)}$, the block effect (random); e_{hijkl}, residual effect (random).

The unconditional genetic effects from different genetic systems were defined as the total accumulated effects of genes expressed from flowering (0) to a particular time (t) during the developmental period. The unconditional genetic main variance ($V_{G(t)}$) comprised of embryo additive variance ($V_{A(t)}$), embryo dominance variance ($V_{D(t)}$), cytoplasmic variance ($V_{C(t)}$), maternal additive variance ($V_{Am(t)}$) and maternal dominance variance ($V_{Dm(t)}$). The unconditional GE

interaction variance ($V_{GE(t)}$) included embryo additive interaction variance ($V_{AE(t)}$), embryo dominance interaction variance ($V_{DE(t)}$), cytoplasm interaction variance ($V_{CE(t)}$), maternal additive interaction variance ($V_{AmE(t)}$) and maternal dominance interaction variance ($V_{DmE(t)}$) at each developmental time t (Zhu and Weir 1994, 1996). The embryo and maternal additive covariance ($C_{A\cdot Am(t)}$) or dominance covariance ($C_{D\cdot Dm(t)}$), embryo and maternal additive interaction covariance ($C_{AE\cdot AmE(t)}$) or dominance interaction covariance ($C_{DE\cdot DmE(t)}$) were estimated, as partial embryo genes derived from maternal plants and the above four covariances might exist between embryo and maternal effects. The unconditional genetic effects from different genetic systems were defined as the total accumulated effects of genes expressed from flowering (0) to a particular developmental time (t). Unconditional phenotypic variance ($V_{P(t)}$) was partitioned as follows:

$$\begin{aligned} V_{P(t)} &= V_{A(t)}+V_{D(t)}+V_{C(t)}+V_{Am(t)}+V_{Dm(t)}+V_{AE(t)}+V_{DE(t)} \\ &\quad + V_{CE(t)}+V_{AmE(t)}+V_{DmE(t)}+2(C_{A\cdot Am(t)}+C_{D\cdot Dm(t)}) \\ &\quad + 2(C_{AE\cdot AmE(t)}+C_{DE\cdot DmE(t)})+V_{e(t)}. \end{aligned}$$

For the conditional analysis, the developmental genetic models and statistical methods developed by Zhu (1995) were used to estimate the conditional genetic variance components at the specific period ($t-1 \rightarrow t$) during seed development (Shi *et al.* 2006). The conditional phenotypic variance ($V_{P(t|t-1)}$) was expressed as follows:

$$\begin{aligned} V_{P(t|t-1)} &= V_{A(t|t-1)}+V_{D(t|t-1)}+V_{C(t|t-1)}+V_{Am(t|t-1)} \\ &\quad + V_{Dm(t|t-1)}+V_{AE(t|t-1)}+V_{DE(t|t-1)} \\ &\quad + V_{CE(t|t-1)}+V_{AmE(t|t-1)}+V_{DmE(t|t-1)} \\ &\quad + 2(C_{A\cdot Am(t|t-1)}+C_{D\cdot Dm(t|t-1)}) \\ &\quad + 2(C_{AE\cdot AmE(t|t-1)}+C_{DE\cdot DmE(t|t-1)})+V_{e(t|t-1)} \end{aligned}$$

Net genetic effects were defined as the effects of gene expression activated in the period $t-1$ to time t ($t-1 \rightarrow t$), were detected by using conditional analysis. In the present experiment, 15d|0d indicated the accumulative effects of gene expression from the initial time to 15 days after flowering and fertilization, while 22d|15d represents the measures at 22nd day given the phenotype values measured at 15th day for conditional analysis, and so on.

The estimated total narrow-sense heritability (h^2) at different developmental times was further differentiated into general heritability (h^2_G) controlled by genetic main effects and interaction heritability (h^2_{GE}) controlled by GE interaction effects. General heritability has the components of embryo general heritability (h^2_{Go}), cytoplasmic general heritability (h^2_{Gc}) and maternal general heritability (h^2_{Gm}), and the interaction heritability has components of embryo interaction heritability (h^2_{GoE}), cytoplasmic interaction heritability (h^2_{GcE}) and maternal interaction heritability (h^2_{GmE}). The genotypic correlation coefficients among different developmental times of rapeseed or between leucine and isoleucine content were separated into genetic main correlations (r_G) including embryo additive correlation (r_A), embryo dominance correlation (r_D), cytoplasm correlation (r_C), maternal additive correlation (r_{Am}), maternal dominance correlation (r_{Dm}), and the GE interaction correlations (r_{GE}) including embryo additive interaction correlation (r_{AE}), embryo dominance interaction correlation (r_{DE}), cytoplasm interaction correlation (r_{CE}), maternal additive interaction correlation (r_{AmE}), dominance interaction correlation (r_{DmE}) (Chen *et al.* 2011a).

The Jackknife resampling methods (Miller 1974; Zhu and Weir 1996) were applied by sampling generation means of entries to derive the standard errors of estimated unconditional or conditional variance, heritability and correlation coefficient components.

Results

Unconditional variance components analysis at different developmental times

Unconditional variance components for leucine and isoleucine contents of rapeseed meal at five developmental times (15, 22, 29, 36 and 43 DAF) are summarized in table 1. It was found that the leucine and isoleucine contents of rapeseed meal were simultaneously controlled by the genetic main effects and GE interaction effects.

Comparing the genetic main effects ($V_{G(t)} = V_{A(t)} + V_{D(t)} + V_{C(t)} + V_{Am(t)} + V_{Dm(t)}$) with GE interaction effects ($V_{GE(t)} = V_{AE(t)} + V_{DE(t)} + V_{CE(t)} + V_{AmE(t)} + V_{DmE(t)}$), the leucine contents of rapeseed at 36 and 43 DAF were mainly affected by the genetic main effects. The $V_{G(t)}$ accounted for 54.26 and 69.06% of total genetic variances at 36 and 43 DAF, respectively. Similarly, isoleucine content at 43 DAF was mainly controlled by the genetic main effects, with $V_{G(t)}$ accounting for 55.84% of total variances.

The GE interaction effects were more important at 15, 22, and 29 DAF with $V_{GE(t)}$ of 69.22, 77.40 and 87.40% of total genetic variance for leucine content. For isoleucine content, the GE interaction effects were also dominant, with estimated $V_{GE(t)}$ of 71.45, 83.28, 87.67 and 51.03% of total genetic variances at 15, 22, 29 and 36 DAF, respectively. This suggested that the genes expression for leucine and isoleucine contents during the developmental process would be more easily influenced by environmental conditions.

Among the embryo, cytoplasmic, and maternal plant genetic systems for leucine content, it was observed that the maternal effects ($(V_{Am(t)}+V_{Dm(t)}+V_{AmE(t)}+V_{DmE(t)})/(V_{G(t)}+V_{GE(t)})$) were more prominent at 15, 22, 29, 36 and 43 DAF, with variable of 64.88, 50.96, 62.17, 54.90 and 61.91% of total genetic variance, respectively, whereas the embryo effects accounted for 27.27–38.09%. Since the cytoplasmic effects ($V_{C(t)}+V_{CE(t)}$) were significant at most developmental times, the sequential expression of genes from cytoplasmic

Table 1. Estimates of unconditional variance components for leucine and isoleucine contents at different developmental stages in rapeseed meal.

Parameter	Leucine content ($\times 10^{-2}$)					Isoleucine content ($\times 10^{-2}$)				
	15 d	22 d	29 d	36 d	43 d	15 d	22 d	29 d	36 d	43 d
$V_{A(t)}$	0.00	0.00	0.00	5.58**	2.12**	0.00	0.00	0.00	1.60**	0.57**
$V_{D(t)}$	4.57**	2.27**	0.00	1.83**	0.80**	1.34**	1.14**	0.00	0.40**	0.26**
$V_{C(t)}$	0.00	5.39**	0.00	0.00	0.00	0.00	1.59**	0.00	0.00	0.00
$V_{Am(t)}$	0.00	0.00	0.00	6.47**	2.66**	0.00	0.00	0.00	1.34**	1.01**
$V_{Dm(t)}$	5.86**	6.22**	4.35**	3.97**	1.72**	1.90**	2.79**	2.71**	0.85**	0.63**
$V_{AE(t)}$	0.00	11.69**	8.93**	0.00	0.00	0.00	8.76**	5.09**	0.00	0.55**
$V_{DE(t)}$	5.67**	2.79**	4.15**	2.90**	1.11**	1.48**	1.01**	2.30**	0.64**	0.28**
$V_{CE(t)}$	1.66**	7.98**	0.00	4.52**	0.00	1.82**	2.93**	2.06**	0.90**	0.40**
$V_{AmE(t)}$	9.65**	18.49**	11.79**	4.06**	0.00	2.98**	12.26**	6.15**	1.74**	0.71**
$V_{DmE(t)}$	6.49**	6.58**	5.34**	3.55**	2.16**	1.82**	2.49**	3.64**	1.08**	0.00**
$C_{A\cdot Am(t)}$	0.00	0.00	0.00	0.26	0.41	0.00	0.00	0.00	0.16	0.50*
$C_{D\cdot Dm(t)}$	−0.94	−0.86	0.00	−0.58	−0.05	−0.35	−0.45	0.00	−0.01	−0.06
$C_{AE\cdot AmE(t)}$	0.00	−2.99	−0.90	0.00	0.00	0.00	−4.36	−0.53	0.00	0.04
$C_{DE\cdot DmE(t)}$	−1.36	0.68	−0.75	−0.56	−0.15	−0.19	0.23	−0.69	−0.16	−0.11
$V_{e(t)}$	1.87**	4.77**	2.20**	1.95**	1.20**	0.89**	1.80**	0.86**	0.47**	0.37**

+Significant at $P < 0.10$. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

genetic system could not be ignored for improving leucine content of rapeseed. The additive variances accounted for 49.15, 59.06 and 48.98% of total genetic variances for leucine content at 22, 29, and 36 DAF while, dominance variances accounted for 66.64 and 54.78% at 15 and 43 DAF suggesting that the additive effects of the genes expression from different genetic systems in most of the developmental times were more crucial for controlling the leucine contents.

For isoleucine content, genetic effects were also found to be mainly controlled by the maternal genetic system, with estimated maternal genetic effects of 59.11, 53.20, 56.96, 58.65 and 53.33% of total genetic variance at five developmental times. To improve isoleucine content, selection based on the performance of maternal plant would be more effective than that of single seed. In addition, embryo genetic system, though low at 24.83–37.63%, also played an important role in the formation of isoleucine content during the whole developmental period. Significant cytoplasmic interaction variances were observed throughout the developmental period. However, cytoplasmic main effect was only exhibited at 22 DAF, which indicated that the expression of cytoplasmic genes was influenced by the environment for isoleucine content in rapeseed. Additive effects were more important than dominance effects for isoleucine content, with significantly higher values of 63.78, 51.20, 54.68 and 64.32% at 22, 29, 36 and 43 days, respectively. Therefore, early generation(s) selection could improve isoleucine content of rapeseed.

Table 1 showed that the unconditional covariance ($C_{A\cdot Am(t)}$, $C_{D\cdot Dm(t)}$, $C_{AE\cdot AmE(t)}$ and $C_{DE\cdot DmE(t)}$) were not significant except for $C_{A\cdot Am(t)}$ at 43 days for isoleucine content. However, there was a notable relationship between unconditional embryo additive effect and unconditional maternal additive effect for the isoleucine content at the same stage. The residual variances at various developmental times were all significant, so the leucine and isoleucine contents could have been significantly affected by sampling errors. Nevertheless, it was speculated that both quality traits of rapeseed were mainly affected by genetic effects because the estimated $V_{e(t)}$ was small.

Conditional variance components analysis at different developmental stages

The results obtained using unconditional variance analysis showed differences in gene expression for leucine and isoleucine contents of rapeseed at different developmental times. However, unconditional variances could only clarify the changes of accumulated genetic effects expressed from flowering to developmental time $t(0\rightarrow t)$, and could not elucidate the gene expression at one specific developmental stage $(t-1\rightarrow t)$. So a conditional analysis approach which can clarify the genes expression at one specific stage is required to understand the dynamic genetic effects at different stages in the whole developmental period. Table 2 shows the variance components estimated using the conditional method.

The results showed that the activation of quantitative genes for leucine and isoleucine contents was gradually carried out through the different developmental stages and there were some diversity for the magnitude or type of conditional genetic effects among the developmental stages. The gene expression at some specific developmental stages was much higher, especially for 1–15 days for leucine and 16–22 days for isoleucine traits, indicating that the embryo,

Table 2. Estimates of conditional variance components for leucine and isoleucine contents at different developmental stages in rapeseed meal.

Parameter	Leucine content ($\times 10^{-2}$)					Isoleucine content ($\times 10^{-2}$)				
	15|0 d	22|15 d	29|22 d	36|29 d	43|36 d	15|0 d	22|15 d	29|22 d	36|29 d	43d|36 d
$V_{A(t\|t-1)}$	0.00	0.00	5.50**	4.00**	1.43**	0.00	0.00	1.95**	1.12**	0.39**
$V_{D(t\|t-1)}$	4.57**	2.03**	0.00	1.54**	0.80**	1.34**	1.06**	0.00	0.37**	0.25**
$V_{C(t\|t-1)}$	0.00	0.00	0.00	2.17**	0.88**	0.00	0.00	0.00	0.52**	0.36**
$V_{Am(t\|t-1)}$	0.00	0.00	0.00	0.93**	1.36**	0.00	0.00	0.00	0.00	0.31**
$V_{Dm(t\|t-1)}$	5.86**	5.43**	4.27**	2.95**	1.54**	1.90**	2.46**	2.54**	0.61**	0.50**
$V_{AE(t\|t-1)}$	0.00	0.00	0.00	0.00	0.00	0.00	4.42**	0.00	1.37**	0.00
$V_{DE(t\|t-1)}$	5.67**	2.59**	3.86**	1.99**	1.06**	1.48**	0.93**	2.36**	0.47**	0.29**
$V_{CE(t\|t-1)}$	1.66**	9.85**	0.00	0.00	0.00	1.82**	2.85**	2.17**	0.00	0.22**
$V_{AmE(t\|t-1)}$	9.65**	0.00	13.94**	5.62**	0.00	2.98**	4.64**	5.98**	1.69**	0.71**
$V_{DmE(t\|t-1)}$	6.49**	5.71**	5.62**	3.23**	2.06**	1.82**	2.33**	3.44**	0.89**	0.42**
$C_{A\cdot Am(t\|t-1)}$	0.00	0.00	0.00	0.48	0.64^{+}	0.00	0.00	0.00	0.00	0.80**
$C_{D\cdot Dm(t\|t-1)}$	−0.94	−0.80	0.00	−0.42	−0.18	−0.35	−0.41	0.00	−0.05	−0.06
$C_{AE\cdot AmE(t\|t-1)}$	0.00	0.00	0.00	0.00	0.00	0.00	−1.05	0.00	−0.39	0.00
$C_{DE\cdot DmE(t\|t-1)}$	−1.36	0.73	−0.89	−0.34	−0.11	−0.19	0.20	−0.78	−0.06	−0.07
$V_{e(t\|t-1)}$	1.87**	4.75**	2.18**	1.80**	1.15**	0.89**	1.79**	0.85**	0.43**	0.37**

$^{+}$Significant at $P < 0.10$. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

cytoplasmic and/or maternal effects of gene expression were operative at the specific stages. Further, the conditional GE interaction variances ($V_{GE(t|t-1)}$) at 15, 22, 29, 36, and 43 DAF accounted for 69.22, 70.87, 70.54, 48.33, 34.14% for leucine content and 71.45, 81.20, 75.65, 62.77, 47.54%, respectively for isoleucine content in total genetic variances ($V_{G(t|t-1)} + V_{GE(t|t-1)}$), the net GE interaction effects from different genetic systems were important for both nutritional quality traits among the specific rapeseed developmental stages except at the mature stage (37–43 days). Hence, gene expression at most of the developmental stages of rapeseed was more sensitive to the environmental conditions.

At different developmental stages, the new expression of maternal nuclear genes ($(V_{Am(t|t-1)}+V_{Dm(t|t-1)}+V_{AmE(t|t-1)}+V_{DmE(t|t-1)})/(V_{G(t|t-1)} + V_{GE(t|t-1)})$) for leucine and isoleucine contents, appeared to be more important than others, except at 30–36 DAF, where the net embryo effects ($(V_{A(t|t-1)} + V_{D(t|t-1)} + V_{AE(t|t-1)} + V_{DE(t|t-1)})/(V_{G(t|t-1)} + V_{GE(t|t-1)})$) were larger, in observed values of 47.35% for isoleucine traits. The net embryo effects ranged from 18.05% to 36.07% for leucine content, and 23.35% to 47.35% for isoleucine content.

For leucine content, significant cytoplasmic effects ($V_{C(t|t-1)} + V_{CE(t|t-1)}$) were observed except for 23–29 DAF. At all the developmental stages, conditional additive and cytoplasmic effects among variance components were found to be more important for both leucine and isoleucine contents.

The theory of developmental genetics emphasizes that numerous quantitative polygenes might be selectively and sequentially expressed during the development of quality traits. In the current study, the results of conditional genetic analysis revealed that $V_{A(15d|0d)}$, $V_{A(22d|15d)}$, $V_{D(29d|22d)}$, $V_{C(15d|0d)}$, $V_{C(22d|15d)}$, $V_{C(29d|22d)}$, $V_{Am(15d|0d)}$, $V_{Am(22d|15d)}$, $V_{Am(29d|22d)}$, $V_{AE(t|t-1)}$, $V_{CE(29d|22d)}$, $V_{CE(36d|29d)}$, $V_{CE(43d|36d)}$, and $V_{Am(22d|15d)}$ for leucine content, and $V_{A(15d|0d)}$, $V_{A(22d|15d)}$, $V_{D(29d|22d)}$, $V_{C(15d|0d)}$, $V_{C(22d|15d)}$, $V_{C(29d|22d)}$, $V_{Am(15d|0d)}$, $V_{Am(22d|15d)}$, $V_{Am(29d|22d)}$, $V_{Am(36d|29d)}$, $V_{AE(15d|0d)}$, $V_{AE(29d|22d)}$, $V_{AE(43d|36d)}$, $V_{CE(36d|29d)}$ for isoleucine content were absent, suggesting that at these specific developmental stages there was no new gene expression from the involved genetic system. The corresponding significant unconditional genetic effects detected in table 1 for some developmental times might be attributed to the durative expression of activated genes at the previous developmental stages. It also suggested that developmental regulatory mechanisms might have affected gene expression at various stages. For example, $V_{CE(36d|29d)}$ was absent for isoleucine content, contrary to unconditional variance analysis.

The results shown in table 2 revealed that the conditional covariances ($C_{A\cdot Am(t|t-1)}$, $C_{D\cdot Dm(t|t-1)}$, $C_{AE\cdot AmE(t|t-1)}$ and $C_{DE\cdot DmE(t|t-1)}$) were not significant except for $C_{A\cdot Am(t|t-1)}$ at 37–43 DAF for isoleucine content. There was a visible relationship between conditional embryo additive effects and conditional maternal additive effects at 37–43 DAF for isoleucine content. In addition, a small but significant conditional residual variance ($V_{e(t|t-1)}$) was observed, indicating that gene expression for leucine and isoleucine contents could be influenced by sampling errors. However, because the estimated $V_{e(t|t-1)}$ was small, it is possible that at various developmental stages both quality traits were mainly affected by genetic effects from different genetic systems.

Heritability components analysis at different developmental times

Since the total genetic effect can be partitioned into different components from genetic main effects and GE interaction effects, the narrow-sense heritability (h^2) can also be further divided into general heritability (h^2_G) and interaction heritability (h^2_{GE}), which have different heritability components from embryo, cytoplasmic and maternal genetic systems.

Table 3 shows estimates of heritabilities for leucine and isoleucine contents. At the first two developmental stages the components of general heritability from the embryo genetic system and maternal plant system were not detected. However, they were detected at 36 and 43 DAF. Cytoplasm general heritability was only found at 22 DAF for both the traits.

Components of maternal interaction heritability were more important at all stages except at 43 DAF, while embryo interaction heritabilities were relatively vital on 22, 29 DAF with significant values of 14.50 and 24.00%. Cytoplasm interaction heritabilities were significant at 15, 22, and 36 DAF. The interaction heritabilities, including embryo, cytoplasm and maternal interaction heritabilities, were larger than their corresponding general heritabilities at 15, 22, and 29 DAF, while general heritabilities were larger at 36 and 43 DAF. The total narrow-sense heritabilities for leucine content at 15, 22, 29, 36 and 43 DAF were 36.30, 62.80, 56.60, 63.90 and 45.90%, respectively. The sum of maternal and cytoplasm general heritabilities and their interaction heritabilities was small at the mature time with 54.80% of h^2, improving leucine content would be difficult when selection is based on maternal plants in early generations.

For isoleucine content, components of interaction heritability for embryo interaction heritabilities were significant except 15 and 36 DAF. The embryo, maternal and cytoplasm interaction heritabilities were larger than components of general heritability at first three developmental times. The total narrow-sense heritabilities at 15, 22, 29, 36, and 43 DAF were 43.0, 65.7, 60.1, 65.5 and 78.2%, respectively. Percentages of maternal and cytoplasm general heritabilities and their interaction heritabilities were relatively higher at all developmental times with values of 100.00, 73.80, 62.80, 70.20 and 61.50%, observed for $(h^2_{Gc}+h^2_{Gm}+h^2_{GcE}+h^2_{GmE})/h^2$. These results suggested that improving isoleucine content would be more efficient when selection is based on maternal plants in early generations.

Genetic correlation components analysis at different developmental times

Analysis of genetic correlation components can be helpful in deciphering the mechanism of interrelation for quality traits of rapeseed among developmental times. The values of genetic correlation components for leucine and isoleucine contents among different developmental times are presented in table 4.

The results revealed that r_{Dm}, r_{DE}, r_{DmE}, and r_{AmE} correlations were more important for leucine content at all developmental times. Significant positive/negative r_{Dm} correlations were observed at early developmental times, whereas significant positive correlations were found near maturity. Significantly negative r_{DE} was observed at early developmental times but positively significant correlations were detected

Table 3. Estimates of heritabilities among different developmental times for leucine and isoleucine contents.

	Developmental times of rapeseed				
Parameter	15 d	22 d	29 d	36 d	43 d
Leucine					
h_2	0.363	0.628	0.566	0.639	0.459
h^2_{Go}	0.000	0.000	0.000	0.177*	0.207*
h^2_{Gc}	0.000	0.090**	0.000	0.000	0.000
h^2_{Gm}	0.000	0.000	0.000	0.203**	0.251*
h^2_{GoE}	0.000	0.145**	0.240$^+$	0.000	0.000
h^2_{GcE}	0.053	0.133**	0.000	0.137**	0.000
h^2_{GmE}	0.309**	0.259**	0.326**	0.123**	0.000
Isoleucine					
h_2	0.430	0.657	0.601	0.655	0.782
h^2_{Go}	0.000	0.000	0.000	0.195*	0.193**
h^2_{Gc}	0.000	0.062**	0.000	0.000	0.000
h^2_{Gm}	0.000	0.000	0.000	0.166*	0.273**
h^2_{GoE}	0.000	0.172	0.224**	0.000	0.108**
h^2_{GcE}	0.163**	0.114**	0.101**	0.100**	0.072**
h^2_{GmE}	0.267**	0.309**	0.276**	0.193**	0.136**

$^+$Significant at $P < 0.10$. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

Table 4. Genetical correlation coefficients among different developmental times for both leucine and isoleucine contents in rapeseed.

	Developmental time of rapeseed									
Parameter	15&22d	15&29d	15&36d	15&43d	22&29d	22&36d	22&43d	29&36d	29&43d	36&43d
Leucine										
r_P	0.03	0.06	0.05$^+$	0.04	0.00	0.03	0.03	0.11**	0.10*	0.11**
r_G	0.04	0.06	0.05	0.03	−0.01	0.03	0.03	0.10*	0.11*	0.10*
r_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26**
r_D	0.02	0.00	0.08$^+$	−0.13**	0.00	−0.09*	0.05	0.00	0.00	−0.06
r_C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
r_{Am}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11**
r_{Dm}	0.16**	−0.01	0.13**	−0.16**	0.00	0.03	−0.03	0.18**	0.17**	0.15**
r_{AE}	0.00	0.00	0.00	0.00	−0.44**	0.00	0.00	0.00	0.00	0.00
r_{DE}	−0.03	−0.11**	0.09*	0.03	0.19**	0.21**	0.09**	0.33**	0.03	0.07$^+$
r_{CE}	0.35**	0.00	0.47**	0.00	0.00	0.10*	0.00	0.00	0.00	0.00
r_{AmE}	0.28**	−0.03	−0.13**	0.00	−0.33**	−0.06	0.00	−0.25**	0.00	0.00
r_{DmE}	−0.27**	0.06$^+$	−0.16**	0.14**	0.03	−0.13**	0.01	0.05	−0.06$^+$	−0.10**
r_e	−0.09	0.01	0.04	0.11$^+$	0.07	0.09	0.03	0.26**	0.07	0.20**
Isoleucine										
r_P	0.02	0.06$^+$	0.03	0.04	0.01	0.02	0.03	0.11**	0.09*	0.14**
r_G	0.03	0.07$^+$	0.04	0.05	0.00	0.02	0.03	0.10*	0.09*	0.14**
r_A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21**
r_D	0.07$^+$	0.00	0.16**	−0.15**	0.00	0.15**	0.01	0.00	0.00	0.03
r_C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
r_{Am}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08$^+$
r_{Dm}	0.10*	−0.03	0.03	−0.02	−0.04	0.03	−0.05	0.12**	0.18**	0.32**
r_{AE}	0.00	0.00	0.00	0.00	−0.30**	0.00	−0.19**	0.00	0.07$^+$	0.00
r_{DE}	−0.18**	−0.08*	−0.05	0.10**	−0.06$^+$	−0.03	0.00	0.28**	0.19**	0.02
r_{CE}	0.03	0.18**	0.21**	−0.21**	−0.23**	0.01	−0.32**	0.16**	−0.06	0.12**
r_{AmE}	0.40**	−0.07$^+$	0.04	−0.04	−0.26**	−0.04	0.11**	−0.31**	−0.09*	0.25**
r_{DmE}	−0.25**	0.12**	0.01	1.00**	0.13**	0.01	−0.12**	0.19**	0.42**	−1.00**
r_e	−0.06	0.03	−0.03	−0.08	0.10$^+$	0.04	0.01	0.27**	0.07	0.12*

$^+$Significant at $P < 0.10$. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

at later times. Most of the maternal additive and dominant interaction correlations were significantly negative except for r_{AmE} between 15 and 29 DAF or r_{DmE} between 15 and 29, and 15 and 43 DAF. It appeared that the embryo and maternal correlations are easily influenced by the environment. Similar results were observed for isoleucine content. The results further showed that most genetic main correlations were not significant. Among the different correlation components, r_{DmE} for isoleucine content were the most important, because their r_{DmE} were significant except those between

Table 5. Correlation between leucine and isoleucine contents at different developmental times.

Parameter	15 d	22 d	29 d	36 d	43 d
r_P	0.151**	0.142**	0.159**	0.156**	0.181**
r_G	0.098*	0.080*	0.120*	0.121**	0.143**
r_A	0.000	0.000	0.000	0.307**	0.164**
r_D	0.233**	0.321**	0.000	0.039	0.122**
r_C	0.000	0.066$^+$	0.000	0.000	0.000
r_{Am}	0.000	0.000	0.000	0.096*	0.124**
r_{Dm}	0.214**	0.327**	0.161**	0.232**	0.295**
r_{AE}	0.000	0.250**	0.025	0.000	0.000
r_{DE}	0.419**	0.124**	0.378**	0.367**	0.268**
r_{CE}	0.505**	0.320**	0.000	0.118**	0.000
r_{AmE}	−0.144**	0.359**	0.154**	−0.051	0.000
r_{DmE}	0.319**	−0.153**	0.275**	0.228**	1.000**
r_e	0.886**	0.923**	0.884**	0.755**	0.621**

$^+$Significant at $P < 0.10$. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

15 and 36 DAF, and 22 and 36 DAF; followed by r_{CE} except between 15 and 22, 22 and 36, and 29 and 43 DAF; and r_{AmE} ranks third behind r_{DmE} and r_{CE} followed by r_{DE}. Therefore considering the developmental times, the correlations including r_{DE}, r_{CE}, r_{DmE} and r_{AmE} were important for isoleucine content. It revealed that the relationships among genetic effects from the embryo, cytoplasm and/or maternal nucleus was time dependent and mainly relied on GE interaction correlations, especially on dominance interaction correlations.

Correlation between leucine and isoleucine contents at different developmental times

Genetic correlation between leucine and isoleucine contents at different developmental times is presented in table 5. The genetic relationship between leucine and isoleucine were more notable at 36 or 43 DAF. Significant positive r_P and r_G were found between leucine and isoleucine at 29, 36 and 43 DAF. Negative correlations were observed for r_{AmE} at 15 DAF and for r_{DmE} at 22 DAF. Since significant genetic correlation components among different genetic systems were mostly positive at different developmental times, it is possible that simultaneous improvement can be achieved for these quality traits.

Discussion

Most nutritional quality traits in crops are controlled by a complex genetic system consisting of major gene and numerous polygenes. The genetic mechanism was closely related to the overall formation of these traits. The developmental genetics theory emphasizes that there exist varieties of gene action and the variations of gene expression might exist throughout individual developmental process. Some genes may be active while others may be in silence. The expression of some genes can be constant, while other genes may be expressed strictly in a temporal and spatial manner at different stages. Polygene system of complex quantitative traits could have specific expression pattern at different developmental stages. A number of previous results showed that many quality traits are formed in a developmental and tissue-specific manner (Cheung *et al.* 1996). The genetic control of complex quantitative traits would also vary with developmental stages and environments (Tan *et al.* 2000). Gao (1994) found that change of the nuclear DNA content of corn embryo was significantly related to the growth of seed weight/volume. This supports to emphasize on the timing of dynamic variation in gene expression for understanding the genetic mechanisms of complex quantitative traits during the developmental process across different environments.

The developmental genetic models and corresponding statistical analysis methods developed by Zhu (1995) could be effectively used to analyse the dynamic variation about the expression of genes at specific stage from $t-1$ to t for the quality traits. In the present study, developmental genetic mechanism for leucine and isoleucine contents of rapeseed was investigated by analysing the accumulative genetic effects at the developmental time ($0\rightarrow t$) and net genetic effects in one specific developmental stage ($t-1\rightarrow t$). The performance of leucine and isoleucine contents at different developmental times/stage of rapeseed was simultaneously controlled by the expression of diploid embryo nuclear genes, cytoplasmic genes, and diploid maternal plant nuclear genes. The results of conditional analysis in the present experiment demonstrated that the activation of quantitative genes for leucine and isoleucine contents of rapeseed was gradually carried out throughout developmental stages. Net genetic effects from different genetic systems were detected at most stages. The expression of genes was more active at initial developmental stage (1–15 days) for leucine content and at early developmental stage (16–22 days) for isoleucine content. Further, the results from conditional analysis revealed that the gene expression in the embryo, cytoplasm and maternal plant genetic systems were essential for the final phenotype of leucine and isoleucine contents. Compared with the conditional genetic variances between two nutritional traits at various developmental stages, it was not difficult to find that the expression of genes controlling the leucine and isoleucine contents in rapeseed meal were different in the whole developmental period. This demonstrated the time sequence of gene expression and relative contribution of different genetic effects for the performance of quantitative trait(s). These results might be useful for quantitative traits loci (QTLs) analysis and marker assisted selection (MAS) for improving leucine and isoleucine contents of rapeseed, by identifying genes specific to various developmental stages.

Plants growing in natural fields are affected by genetic factors and environmental conditions. During this study, the overall temperature was higher in 2008 than in 2007 at the experimental field. There was adequate sunshine and a clear differences in illumination were observed during growing period in 2008. The climatic conditions and management differences between the two environments (years) might be the possible reason for interpretation of variation of gene expression on leucine and isoleucine contents of rapeseed meal. Therefore, it is better for breeders to pay more attention for the variation of rapeseed quantitative traits across environments and conduct the experiments in different places or years. There existed differences of GE interaction effects for formation of quality traits during different developmental stages. In this experiment, the results from unconditional and conditional variance analyses revealed that the GE interaction effects were more important than the genetic main effects for leucine content at first three developmental stages while, the contrary was true at later two stages of rapeseed, and for isoleucine content at first four developmental stages while the genetic main effects was larger at mature stages of rapeseed. The GE interaction effects at different

developmental stages indicated that the sequential expression of genes in embryo, cytoplasm and maternal plant was influenced with time by environmental conditions.

In quality breeding programme, a new rapeseed variety would be required with better quality traits. Now breeders aim at the genetic improvement of multiple traits in the selection, knowing genetic correlations among important quality traits besides understanding inheritance of quality traits. Complex correlations exist among some quality traits of rapeseed because of genetic linkage and the 'pleiotropic' function of genes, causing difficulties in selecting better genotypes from subsequent generations. Genotypic correlation could eliminate the interference caused by random error, and is an important statistical parameter that is superior to phenotypic correlation. Since genotypic correlation is the general relationships including all genetic effects between the two traits, the indirect selection based on genotypic correlation sometimes does not acquire the ideal results, especially in the early generations because of the influence of dominance correlation(s). In addition, the performance of some quality traits of rapeseed have been found to be simultaneously affected by genetic main effects from embryo, cytoplasm and maternal plant as well as their GE interaction effects (Shi *et al.* 2003; Zhang *et al.* 2004a, b; Wu *et al.* 2005). The genotypic correlation of the quality traits could also be divided into components of genetic main correlation controlled by genetic main effects and GE interaction correlation resulting from GE interaction effects across different genetic systems. The genetic correlation components from different genetic systems could be applied to dissect the genetic association among different quality traits of rapeseed and used for improving of desired pairwise traits. In the present experiment, most components of genetic main correlation and GE interaction correlation between leucine and isoleucine contents of rapeseed were significantly positive at various developmental times, especially for embryo or maternal dominance interaction correlations, which indicated that the activation and expression of genes between different times or the quality traits had close relationships (Chen *et al.* 2011a). Correlation analysis among different developmental times tends to broaden our understanding of the interrelationships for quality traits.

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